Confirming Vertical Fetal Infection With Coronavirus Disease 2019

Neonatal and Pathology Criteria for Early Onset and Transplacental Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 From Infected Pregnant Mothers

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• Increasing numbers of pregnant women with coronavirus disease 2019 are being reported around the world. The majority of neonates delivered to pregnant women infected with severe acute respiratory syndrome coronavirus 2 have been negative for the virus, but a small number have tested positive for infection. It is important to determine whether vertical transmission of coronavirus disease 2019 occurs and the mechanisms for its development. Based on a number of clinical and laboratory findings, it has been suggested that transplacental transmission may be occurring, but a method to confirm this is necessary. This communication analyzes and evaluates the covariables that have been discussed as potential indicators of vertical and, specifically, intrauterine transmission, including the timing of onset of neonatal illness, neonatal viral test positivity, neonatal antibody testing for immunoglobulin (Ig) G and IgM, and viral analysis of swabs of whole specimens of placental tissue. None of these methods can provide confirmatory evidence that infection developed prior to labor and delivery, or that transplacental transmission occurred. This commentary proposes that diagnosis of early-onset neonatal coronavirus disease 2019 infection should be limited to neonates with positive reverse transcription polymerase chain reaction testing for severe acute respiratory syndrome coronavirus 2 within the initial 72 hours of life. It also proposes that the occurrence of intrauterine transplacental severe acute respiratory syndrome coronavirus 2 among infected mother-infant dyads be based upon identification of severe acute respiratory syndrome coronavirus 2 in chorionic villus cells using immunohistochemistry or nucleic acid methods such as in situ hybridization. Evaluating placentas from neonates with coronavirus disease 2019 using these methods will be instrumental in determining the potential role and prevalence of transplacental transmission of the coronavirus.

(*Arch Pathol Lab Med*. 2020;144:1451–1456; doi: 10.5858/arpa.2020-0442-SA)

Following the introduction of a new virus into the human population, among the most important questions that typically arise are what its effect will be on pregnant women and whether it will be transmissible to the developing fetus. This has been true with many emerging viral agents, including hemorrhagic fever agents such as the filoviruses (Ebola and Marburg viruses) and arenaviruses (Lassa virus), retroviruses (HIV), hepeviruses (hepatitis E virus), and, most recently, flaviviruses (Zika virus). All of these emerging viral infections have been shown to be capable of intrauterine maternal-fetal transmission, often, and in some cases typically, with disastrous results. In addition, 2 pathogenic coronaviruses that were first identified in the last 20 years have also caused this concern: the severe acute respiratory syndrome coronavirus, causing severe acute respiratory syndrome, and Middle East respiratory syndrome coronavirus, causing Middle East respiratory syndrome. Although these 2 coronaviruses resulted in maternal morbidity and mortality as well as perinatal deaths, there were fortunately no confirmed cases of intrauterine transmission identified.[1,2]

In December 2019 a new coronavirus was identified in Wuhan, Hubei Province, China. It was the seventh pathogenic member of the family *Coronaviridae* to be reported to cause human disease. This new virus, termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and producing a disease termed coronavirus disease 2019 (COVID-19), quickly spread from Wuhan throughout China, then to neighboring countries, and ultimately throughout the world, and was declared to be a pandemic by the World Health Organization on March 11, 2020. One of the major questions that arose early in the pandemic and

Accepted for publication July 16, 2020.
Published online July 23, 2020.

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The authors have no relevant financial interest in the products or companies described in this article.

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has continued to be of significance is the ability of the virus to be transmitted from the mother to her infant, termed vertical transmission. In particular, it is important to understand whether SARS-CoV-2 is being transmitted in utero via the transplacental route. This communication examines clinical, laboratory, and pathology methods for determining whether transplacental maternal-fetal transmission of COVID-19 is occurring, and how this process can be evaluated and ultimately confirmed.

MECHANISMS OF VERTICAL TRANSMISSION

Viruses can be vertically transmitted from mother to infant through 3 different mechanisms: intrauterine, intrapartum, and postpartum routes.[3] Intrauterine viral transmission can occur via 2 major mechanisms: the hematogenous route and the ascending route. The hematogenous route is characteristic of most mother-to-fetus–transmissible viral agents. In this mechanism, the virus is circulating in the maternal bloodstream during pregnancy, enters the placenta via maternal blood perfusing the placenta through the uterine arterioles, and crosses the maternal-placental interface to reach the fetal vessels in the chorionic villus tree and be transmitted through the umbilical blood vessels to the fetus.[4,5] Hematogenous vertical transmission occurs with such viruses as rubella, cytomegalovirus, parvovirus, Zika virus, and Ebola virus, and requires that the virus be present in the maternal blood—viremia. The ascending route of intrauterine fetal infection occurs when microorganisms present in the lower genital tract ascend the cervicovaginal tract to reach the pregnant uterine cavity, from where they breach the placental membranes and infect the amniotic fluid. This mechanism almost always is the result of a bacterial infection.[3]

Intrapartum transmission occurs around the time of labor and delivery when the fetus passes through an infected birth canal during vaginal delivery. This type of vertical transmission can occur with herpes simplex virus[6,7]; intrapartum transmission causes 85% of vertical herpes simplex virus infections and is the basis for performing cesarean delivery in infected mothers. Human papillomavirus and HIV can also be transmitted to the infant through intrapartum exposure during labor and delivery.[8,9]

Postpartum vertical transmission of viruses develops following delivery. It can occur through contaminative transmission of a virus from an infected mother via respiratory secretions and fomites, skin-to-skin contact, and breast milk. Respiratory viruses may be transmitted by this mechanism, as well as those viral agents present in breast milk, including HIV,[10] cytomegalovirus,[11] Ebola virus,[12] and others.

The large majority of infants born to pregnant women with COVID-19 have been uninfected in China, the United States, Europe, and all other regions where the pandemic is occurring.[13–19] The low rate of neonatal infection could be expected based on data of vertical transmission existing with other RNA respiratory viruses, including the coronaviruses severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus[1] as well as influenza, respiratory syncytial virus, parainfluenza, and human metapneumovirus.[1,2] The factors inherent in the observed inhibition of respiratory RNA viruses from undergoing intrauterine vertical transmission result from both viral and host factors, and involve the ability of the virus to penetrate the maternal-fetal interface including the placenta, avoiding the innate immune system, and tropism of the virus to host cells.[2]

Among the reported neonates who have had positive tests for COVID-19, some have been asymptomatic,[20–23] while other neonates have had symptomatic illness.[22,24] Among symptomatic infants testing positive for COVID-19, there has been variation in the onset of illness, ranging from within 1 or 2 hours after delivery to within the first 24 to 72 hours of life and up to many days to weeks following birth.[13,23–25] Some of these cases have been considered to be suspicious for vertical transmission, but the mechanisms and timing for the neonate's acquiring the infection have remained unknown.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM THE ONSET OF NEONATAL ILLNESS

There is no agreed-upon definition of early-onset neonatal COVID-19 infection. For bacterial infections such as group B streptococcus and *Escherichia coli*, early-onset neonatal sepsis is based upon timing of onset of either bacteremia or bacterial meningitis, which in term infants is less than 7 days of life. In preterm infants in the neonatal intensive care unit, early-onset neonatal sepsis is defined as occurring in the first 72 hours of life, and is generally believed to be the result of bacterial agents transmitted vertically from mother to infant before or during delivery.[26,27] The majority (80%–90%) of early-onset neonatal sepsis bacterial infections clinically present in the initial 24 to 48 hours following delivery.[28]

Zeng et al[22] reported that among 33 neonates whose mothers had COVID-19 infection in Wuhan, China, there were 3 delivered by cesarean section who developed fever and pneumonia and tested positive for the virus on the second day of life. Although it might appear reasonable to correlate the early onset of symptoms with vertical SARS-CoV-2 infection and even intrauterine maternal-fetal transmission in these cases and others, there are problems with this assumption that would make it speculative for several reasons.[17] These include (1) symptoms such as neonatal respiratory distress or pneumonia are not specific to COVID-19—they can have multiple causes, and because preterm delivery is present in between 37% and 63% of infected neonates, it may have a noninfectious etiology that is related to prematurity or is caused by infection by other agents; (2) even in those cases where neonatal pneumonia has radiographic features consistent with COVID-19 pulmonary disease, its presence cannot be construed to result specifically from transplacental viral transmission; (3) the incubation period of COVID-19 following an initial fetal or perinatal exposure to the virus is unknown; and (4) the exact mechanism of transmission of SARS-CoV-2—transplacental versus intrapartum—cannot be reliably distinguished by either the presence or absence of neonatal symptoms in a neonate with a positive reverse transcription (RT) polymerase chain reaction (PCR) test for SARS-CoV-2.[29–33] Thus, the onset of symptoms shortly after birth in a neonate with early positive testing for COVID-19 may be suggestive of vertical transmission, but does not confirm a transplacental mechanism.

Determining Transplacental Transmission From Timing of Neonatal Viral Testing by RT-PCR

The current gold standard for diagnosis of COVID-19 in neonates is by RT-PCR analysis of specimens from

nasopharyngeal (NP) swabs and, in lesser numbers of infants, from swabs of the oropharynx. However, many factors can affect the sensitivity (ability to detect a positive case) and specificity (ability to determine a negative case) of these tests. Reverse transcription polymerase chain reaction analysis has many advantages for testing among children and adults, but its efficacy for neonatal diagnosis has not been established. Both analytical and external factors can have a detrimental effect on the accuracy of RT-PCR results. Preanalytical factors are the major source of errors in laboratory testing and include problems due to the specimen source, sampling methods, timing of sampling, sample storage and transport, and presence of interfering substances.[34] The performance of diagnostic kits has been reported in some cases to be suboptimal.[34] In adult patients, false-negative results have been demonstrated to be another problem with RT-PCR.[35,36]

The Centers for Disease Control and Prevention[37] recommends using NP swabs for molecular testing because in most patients the major mechanisms of transmission of COVID-19 are via the respiratory route and the nasopharynx appears to have the highest concentration of virus. However, in neonates, this has not been definitively established. In fact, when the virus first appears in the nasopharynx of neonates who are infected with SARS-CoV-2 remains unknown. The mechanism(s) of NP colonization by SARS-CoV-2 in cases of potential intrauterine or intrapartum fetal infection also has not been determined, especially in cases where the fetus may become infected hematogenously via transplacental infection.

There has been significant variation in the timing of SARS-CoV-2 positivity for NP and oropharyngeal specimens taken from neonates. In some cases,[24,38,39] specimens taken shortly following birth were initially negative, to subsequently become positive days or even weeks later. In other cases,[24,40] initial testing was positive in the late neonatal period. Early positive testing for COVID-19 of neonates from infected mothers has also been reported,[17,22] in some cases with positive results obtained from specimens taken immediately or within a few hours after birth.[24,39] In those cases of early test positivity, the neonates were delivered by cesarean section, and as a result the possibility of intrauterine vertical transmission remains high. However, it cannot be confirmed based upon the information available.

In a review of 179 newborn infants delivered to women with COVID-19 during the third trimester, SARS-CoV-2 was detected from NP swabs in 6 infants—1 at 16 hours, 2 at 36 hours, and 3 at 48 hours following delivery—with the authors stating that the timing of transmission could not be determined in these cases.[41]

We propose that a neonate having a positive test for SARS-CoV-2 in the initial 72 hours of life be considered to have early-onset COVID-19 infection; it is this group of neonates who are most suspicious for having acquired COVID-19 from vertical transmission prior to or around the period of delivery. The likelihood is even greater when the initial testing is positive sooner—either immediately or within hours after birth. To address this, we also propose the term *very early-onset COVID-19 infection* for infants with test positivity within the initial 24 hours of life. However, testing positive for SARS-CoV-2 within these time frames does not determine whether infection occurred via the transplacental route.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM NEONATAL ANTIBODY ANALYSIS

Among all 5 classes of antibodies, immunoglobulin (Ig) G is the only antibody class with the capability to cross the placenta from the maternal to the fetal circulation, providing passive immunity to the developing fetus and neonate. As a result, the occurrence of elevated levels of IgG in the newborn circulation following delivery is not useful for diagnosing the acute onset of neonatal infection. Because IgM does not cross the placenta, the concept of using postpartum elevated levels of this antibody class to suggest the possibility of intrauterine infections in neonates was initially introduced in the 1960s.[42,43] Currently, the majority of congenital infections are not diagnosed using neonatal IgM levels for several reasons. These include the propensity for IgM assays to have reliability problems, including both false-positive and false-negative results as well as cross-reactivity and testing challenges, as well as technological advances that have been made in the molecular diagnosis of infectious diseases that have very high levels of sensitivity and specificity.[44,45]

There have been several neonates delivered from SARS-CoV-2–infected mothers who have had elevated levels of specific IgG and IgM antibodies detected shortly after birth. In one case,[46] a newborn was found to have developed IgM and IgG specific antibodies to SARS-CoV-2 together with elevated cytokines when tested 2 hours following delivery, but multiple NP swabs from the infant were negative for the virus. In another report,[22] 2 neonates delivered to mothers with COVID-19 were found to have levels of both IgG and IgM that were higher than normal; both infants had NP swabs that tested negative for COVID-19 by RT-PCR. These cases demonstrate that using elevated IgM levels to diagnose neonatal COVID-19, or to surmise that intrauterine infection has occurred, is not highly reliable.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM VIRAL ANALYSIS OF SWABS OR HOMOGENIZED WHOLE SAMPLES OF PLACENTA

The placenta is a unique heterogenous organ: it is composed of cells from 2 genetically distinct individuals. It is also distinctive in possessing a dual blood supply from 2 individuals: the maternal circulation enters the placenta through numerous uterine spiral arterioles and circulates within the intervillous space, and the fetal circulation enters the placenta through the umbilical arteries and circulates throughout all levels of the chorionic villus tree. These 2 circulations are in close contact with one another, but remain separated by a highly specialized trophoblastic barrier. In addition, both maternal and fetal cells are present at the maternal-fetal interface. As a result, a sample of placental tissue removed using a swab, needle, or excisional biopsy contains both maternal and fetal cells, including maternal red and white blood cells in the intervillous space. Following homogenization of the tissue prior to nucleic acid extraction, the maternal and fetal cells become mixed. A positive PCR test for an infectious agent on a homogenized sample of placental tissue or a swab of its contents establishes that the agent is present in the sample, but cannot precisely localize the agent to being in the maternal component, the fetal component, or both. This is illustrated in a recent communication[47] in which swabs and biopsies were taken from the placenta of a stillborn infant delivered to a mother with COVID-19. These specimens were

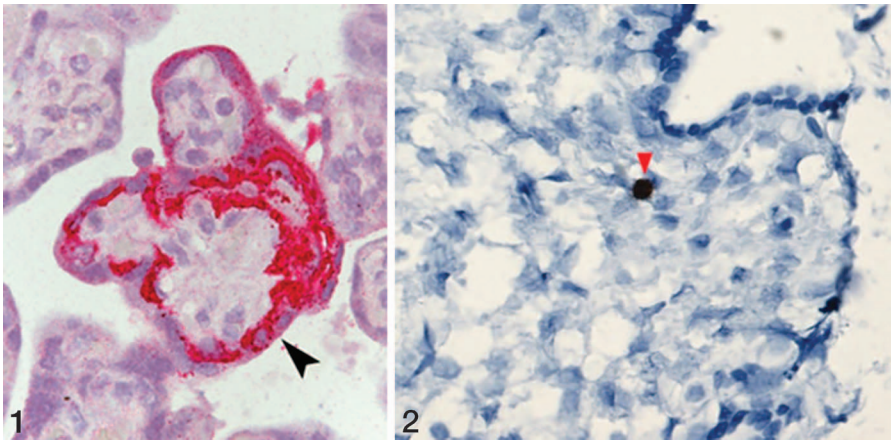

Figure 1. *The placenta from a woman having Ebola virus disease during pregnancy. Both she and her newborn infant died from the infection. Immunohistochemical analysis demonstrates Ebola virus antigen in trophoblast (black arrow). This case and the methodology for immunohistochemical staining were described by the Centers for Disease Control and Prevention in Muehlenbachs et al*[50] *(immunohistochemistry using rabbit polyclonal antisera against Ebola virus, Sudan virus, and Reston virus and Ebola virus hyperimmune mouse ascitic fluid, original magnification ×100).*

Figure 2. *Zika virus RNA (red arrow) is positive by RNAscope in a stromal cell, presumably a Hofbauer cell, in the chorionic villus of the placenta of a preterm infant with congenital Zika virus infection and microcephaly (in situ hybridization for Zika virus, original magnification ×100).*

diagnosed using RT-PCR as being positive for SARS-CoV-2; however, because the samples included cells of both maternal and fetal origin, it was not possible to confirm whether the coronavirus was in cells from the mother or the fetus. Interestingly, despite the placental specimens testing positive for COVID-19, amniotic fluid, maternal vaginal swabs, and all specimens from the fetal autopsy, including swabs from the mouth, axillae, meconium, and fetal blood, obtained within minutes of birth were negative for SARS-CoV-2 by RT-PCR testing. In another report,[48] the amnionic surface of the placenta was swabbed and found to be positive for SARS-CoV-2 following delivery by an asymptomatic mother with COVID-19. The neonate was asymptomatic and uninfected. Thus, this form of placental sampling and PCR analysis cannot reliably be used for confirmation of transplacental viral transmission.

DETERMINING TRANSPLACENTAL TRANSMISSION FROM ANATOMIC LOCALIZATION OF VIRAL SIGNAL IN PLACENTAL TISSUE SECTIONS USING IN SITU PCR OR IMMUNOHISTOCHEMISTRY

Pathology of the placenta, the largest of fetal organs, has been instrumental in understanding the mechanisms of transmission of many different infectious agents from pregnant women to the fetus. Techniques such as immunohistochemistry using antibodies to viral antigens and nucleic acid techniques such as in situ hybridization and RNAscope (Advanced Cell Diagnostics, Newark, California) that detect target RNA molecules within intact cells have the advantage of identifying virus within specific cell types in defined anatomic compartments of the placenta.[49,50] As a result, these techniques can definitively localize a virus to such fetal cells as the syncytiotrophoblast, Hofbauer cells (fetal-derived villous stromal macrophages), extravillous trophoblast, and chorionic villous endothelial cells. These methods have been successfully used in past epidemics to identify and confirm transplacental maternal-fetal viral infection. In cases where both mother and neonate are found to be infected, the placental finding of virus in such chorionic villus cell types as syncytiotrophoblast using immunohistochemistry for Ebola virus (Figure 1) or in Hofbauer cells using an RNAscope in situ hybridization methodology for Zika virus (Figure 2) can confirm transplacental transmission of the pathogen.

The use of in situ hybridization was recently used to identify and localize SARS-CoV-2 in the placentas from 2 infected neonates from Italy. In a study[39] of 22 pregnant women with COVID-19 at an Italian hospital, 2 neonates were found to have positive NP swabs for SARS-CoV-2. Placentas from both infants demonstrated chronic intervillositis, which was accompanied by the presence of CD-68–positive macrophages in both the intervillous and the villous space. The placental tissues were evaluated using in situ hybridization with RNAscope technology, a method that enables the detection of the SARS-CoV-2 spike protein mRNA by using the V-nCoV2019-S probe (Advanced Cell Diagnostics). In both placentas this methodology demonstrated the presence of the coronavirus in the syncytiotrophoblast, indicating the presence of SARS-CoV-2 on the fetal side of the placenta (Figure 3), and proving that

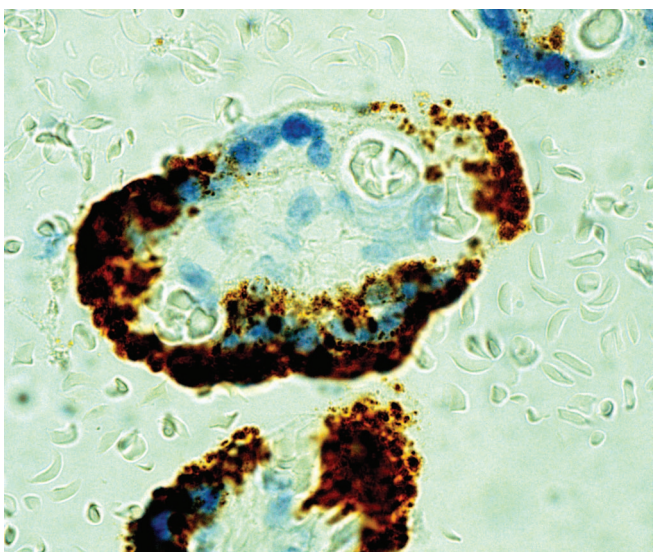

Figure 3. *Brown dots represent positive signals from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein mRNA in this tissue section from a neonate with SARS-CoV-2 infection following delivery from a mother with coronavirus disease 2019. The pattern of RNAscope assay positivity confirms coronavirus infection of the syncytiotrophoblast. This case and the methodology for RNAscope staining were described by Patanè et al*[39] *(in situ hybridization for SARS-CoV-2, original magnification ×100).*

intrauterine infection of fetal cells within the placenta had occurred in the intrauterine environment and prior to delivery. This was the first demonstration of SARS-CoV-2 in chorionic villus tissue of the placenta of infected neonates, establishing intrauterine fetal exposure and infection with the coronavirus.

This communication proposes that the identification of virus in chorionic villus tissue of the placenta, either using in situ nucleic acid hybridization methods as performed by Patanè and colleagues[39] or by immunohistochemistry using virus-specific antibodies to detect viral antigen, provides prima facie evidence of intrauterine fetal infection in the appropriate clinical setting.

CONCLUSIONS

Approximately 217 neonates delivered to pregnant women with COVID-19 have been reported in the literature.[51] More recently, 19 additional infected neonates were reported[24] from 10 hospitals in Iran, among whom there were infants with early and late positive testing for the virus. The relative risks and proportions of neonatal infections that result from a) intrauterine transmission of SARS-CoV-2 from either transplacental or ascending infection; b) acquiring infection during labor and delivery; or c) following delivery from the mother, other individuals, or the environment remain unknown. This knowledge is of critical importance—it can guide management of pregnant mothers with COVID-19, delivery, and postpartum care of the neonate in order to minimize the risk of neonatal infection. In addition, identifying mechanisms of neonatal COVID-19 infection will be useful in determining such factors as the need for cesarean delivery, neonatal isolation in intensive care, guidelines for neonatal resuscitation, safety of rooming-in, avoidance of skin-to-skin contact and breastfeeding, and postpartum contact between the neonate and family members and other individuals. Until these data are established, there will continue to be variation in the care of pregnant women with COVID-19 and their neonates. Institutions, hospitals, professional organizations, and the public health community have attempted to use the limited data available to provide recommendations for clinical care that reduces risk of neonatal infection within the contexts of local social and clinical settings.[50]

In determining whether intrauterine vertical transmission of SARS-CoV-2 has occurred, multiple findings are suggestive; these include positive RT-PCR testing of a neonate for the virus at or shortly after birth, early onset of symptoms, and elevated levels of specific IgM antibodies following delivery. However, confirmation of intrauterine transplacental transmission of COVID-19 should be reserved for those neonates who have demonstrable identification of viral antigen or RNA in fetal-derived placental cells following microscopic pathology testing of tissue sections. These methods include using immunohistochemistry for identification of viral antigen and detection of viral RNA using in situ hybridization or RNAscope methods. It is clear that many, if not most, pathology laboratories will not have the capability of performing these types of tests on placentas. However, it is suggested that whenever feasible, placentas be retained from those neonates suspected of having early-onset COVID-19 and, when necessary, have formalin-fixed tissues sent to appropriate pathology laboratories for special testing and evaluation of transplacental infection. At this point in time, fortunately, it appears as though bona fide transplacental transmission is highly unusual.[52]

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